SEPARATION OF ESSENTIAL OIL FROM MIXTURES OF *MELISSA OFFICINALIS* AND *CYMBOPOGAN CITRATUS* LEAVES FOR MOSQUITO REPELLENT

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ABSTRACT

Synthetic based chemicals such as DEET is one of the most commercialised synthetic based mosquito repellents. This compound is effective against a wide range of insects and arthropods but its usage has raised several concerns to human health and surrounding environment.To combat its negative effects, there has been an overwhelming interest of producing essential oil products from different species of natural plant materials in order to access their repellent properties.Essential oils are odourless volatile mixture of hydrocarbons and their repellent properties have been linked to the presence of monoterpenes and sesquiterpenes.Therefore, it is important to identify a best technique to extract the essential oil without causing significant effect on their chemical constituents, since the conventional methods implied years back are time consuming and also record a low extraction yield.In order to enhance the extraction yield, an improvised approach namely Ultrasound Assisted Extraction-Hydrodistillation method will be exhibited to separate essential oil from mixtures of *Mellissa officinalis* and *Cymbopogan citratus* leaves. At the same time to evaluate the effectiveness of repelling properties in the essential oil, mixtures of leaves have been chosen in this extraction process.In this study, the effects of three main factors which are ultrasonic pre-treatment time, raw material to water ratio, and ultrasonic frequency were investigated.As a result, the best condition gives the highest yields of oil (0.3820%) were found at the ultrasonic frequency of 5kHz, pre-treatment time of 60 min and solid to water ratio of 1:6. The mixture of*Melissa officinalis* and *Cymbopogan citratus* leaves reflects higher essential oil yield compared with the yield of *Mellissa officinalis*leaves. Finally the oil samples were analysed by using Gas Chromatography Mass Spectrometry (GC-MS). From the analysis, almost similar chemical compounds with potentiating repellent activity such as linalool, citronellal, geraniol, citral, α-pinene, and limonene were found in their respective essential oils. Anyhow, the amount it contains differs from one another. Moreover, the repellency test carried out using the extracted oil against mosquito have also showed positive outcome.

ABSTRAK

DEET merupakan salah satu penghalau nyamuk yang dihasilkan berasaskan bahan sintetik dan paling dikomersilkan dalam pasaran sehingga masa kini.Kompaun ini amat berkesan digunakan terhadap pelbagai jenis serangga.Walaubagaimanapun, penggunaannya memberi kesan negatif kepada manusia serta alam sekitar di sekeliling kita. Dalam usaha untuk memerangi kelemahan tersebut, kebelakangan ini carian yang meluas menggunakan bahan berasaskan tumbuhan dijalankan.Justeru, pengeluaran produk minyak pati daripada spesies yang berbeza daripada bahan tumbuhan semulajadi untuk tujuan mengakses ciri-ciri penghalau nyamuk telah mendapat tumpuan orang ramai.Minyak pati adalah campuran menentu hidrokarbon, tidak berbau dan ciri-ciri penghalau mereka telah dikaitkan dengan kehadiran monoterpena dan sesquiterpenes. Oleh itu, adalah penting untuk mengenal pasti teknik terbaik untuk mengekstrak minyak pati tanpa menyebabkan kesan sampingan ke atas kandungan bahan kimia.Hal ini sedemikian kerana kaedah konvensional yang diaplikasikan bertahun –tahun lepas memakan masa dan juga mencatatkan kadar pengeluaran minyak pati yang rendah. Dalam usaha untuk meningkatkan hasil pengeluaran minyak pati , pendekatan yang terbaru iaitu kaedah rawatan pengekstrakan ultrasonik hidro-penyulingan akan digunapakai untuk mengekstrak minyak pati daripada campuran daun *Mellissa officinalis* dan *Cymbopogan citratus*.Dalam kajian ini,campuran daun *Mellissaofficinalis* dan *Cymbopogan citratus* telah dipilih untuk menilai keberkesanan ciri-ciri penghalau dalam minyak pati. Dalam masa yang sama kesan daripada tiga faktor utama iaitu masa pra - rawatan ultrasonik , bahan mentah kepada nisbah air , dan frekuensi ultrasonik telah dikenal pasti. Hasil kajian menunjukkan bahawa keadaan terbaik yang memberikan hasil minyak pati yang paling tinggi adalah pada kekerapan 5kHz , dengan masa pra - rawatan 60 minit dan pepejal kepada nisbah air 1:6.Campuran *Melissa officinalis* dan *Cymbopogan citratus* menggambarkan hasil minyak yang lebih tinggi berbanding dengan hasil *Mellissa officinalis* sahaja.Keputusan ini adalah berdasarkan kepada hasil tertinggi (0.3820 %) yang diperolehi dengan menggunakan campuran daun.Hasil analisis dengan menggunakan kaedah Gas Chromatography Mass Spektrometri (GC-MS)menunjukkan bahawa sebatian kimia yang hampir sama dengan aktiviti penghalau seperti linalool, citronellol, geraniol, citral, α - Pinene, dan limonene telah dikenal pasti. Walau bagaimanapun, jumlah kandungannya berbeza daripada satu

sama lain. Tambahan pula, ujian menghalau nyamuk yang dilakukan dengan menggunakan minyak yang diekstrak terhadap nyamuk juga telah menunjukkan hasil yang positif .

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^{o}C	degree celcius
kHz	kilohertz
min	minutes
g	grams

LIST OF ABBREVIATIONS

UAE-HD	Ultrasonic assisted extraction- Hydrodistillation
EO	Essential oil
HD	Hydro-distillation
UAE	Ultrasonic assisted hydrodistillation
PSE	Pressurized Solvent Extraction
MAE	Microwave Assisted Extraction
SFE	Supercritical Fluid Extraction

1 INTRODUCTION

1.1 Motivation and statement of problem

Insect transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million of person annually (Taubis, 2000).Institute from Medical Research (2013), Ministry Of Health Malaysia has recorded that currently in Malaysia there are 5 human diseases transmitted by mosquitoes and they are namely Malaria, Dengue, Filariasis,Japanese Encophalitis and Chikungunya.According to the latest estimates released in December 2013, there were about 207 million cases of malaria in 2012 (with an uncertainty range of 135 million to 287 million) and an estimated 627 000 deaths (with an uncertainty range of 473 000 to 789 000).Most deaths occur among children living in Africa where a child dies every minute from malaria(WHO,2014).Although personal protective measures such as the use of repellents are widely used to prevent the transmission of arthropod-borne diseases, yet its usage has raised several concern related to environment and human health.

The most common mosquito repellent available in the market are based on DEET(N,N-diethyl-3.methylbenzamide) as it is not only a broad spectrum repellent but also the most effective and persistent on skin(Isman,2006).However, according to Qui et al.(1998) human toxicity reactions after the application of DEET varies from mild to severe. To avoid these adverse effects, there has been an increase in search efforts over the years for a natural and eco-friendly repellent. From the screening done, it has shown that quite a number of essential oil derived from plant species acts as a potential source of repellent and insecticides(Ayanoglu et al., 2005) to replace DEET.

A large number of EO extracted from different families has been shown to have high repellency against arthropod species.Among EO producing plants, some genus such as *Cymbopogon* spp., *Eucalyptus* spp. and *Ocimum* spp. have been widely studied(Luz ,2010).According to Jeanson et al. (2006),repellent properties of several essential oil appear to be linked with the presence of monoterpenes and sesquiterpenes.

Monoterpenes 'such as α-pinene, limonene, terpinolene, citronellal, citronellol, camphor and thymol are common constitutes which presents in mosquito repellent activities(Parket al.,2008).

There are several conventional methods which have been implied to extract essential oil from plant material. However those approaches are not reliable as the extraction times are long, potential loss of volatile constituents, high energy useand yield of oil produced is low. Hence, to improvise the separation process new extraction techniques such as ultrasonic assisted extraction have been applied to shorten extraction time, reduce organic solvent consumption, improve extraction yield and enhance extract quality(Hong, 2010).Mild ultrasonic stressing is introduced so that the desired components which are localised on plant material will ease the separation process (Toma et al., 2001). Therefore, in this research ultrasonic assisted extraction-hydrodistillation (UAE-HD) technique will be implied to evaluate its extraction process using a mixture of *Melissa Officialis* and *Cymbopogan Citratus*leaves. These mixtures of leaves are chosen as an alternative for synthetic based repellent (DEET) since their extracts containpotentiating insecticidal and therapeutic properties as reported by Adeniran et al.(2012).

1.2 Objectives

Thus, the objectives of this research are:

- To separate essential oil from mixtures of *Melissa O.* and *Cymbopogan C.* leaves using Ultrasonic Assisted Extraction – Hydrodistillation (UAE-HD) method.
- To investigate the effects of pre-treatment time, raw material to water ratio and ultrasonic frequency for a maximum oil yield.

1.3 Scope of this research

The following are the scopes which have been identified:

I. Separation of essential oil from *Melissa officinalis* and *Cymbopogan citratus* leaves by using ultrasonic assisted extraction-hydrodistillation (UAE-HD) method. In this process, essential oil will be separated first from *Melissa officinalis* leaves and only then from the mixtures of both leaves. This is done to compare the yield of oil between both leaves and determine the optimum parameters for maximum oil yield.

II. Determination of physical properties of essential oil and characterization of essential oil by using Gas Chromatography Mass Spectrometry (GC-MS) method.

III. Investigation of the effects of pre-treatment time, solid to solvent ratio and ultrasonic frequency on the maximum yield of oil. Graphs of yield against the parameters will be plotted.

IV. Repellence test against mosquito will be carried out.

1.4 Main contribution of this work

The main contribution of this work is to provide a new approach which combines conventional method together with a new technique in order to attain a maximum extraction yield. Besides that, a substitute to overcome the adverse effects of synthetic based repellents on users and to our surrounding environment is also presented.

1.5 Organisation of this thesis

The structure of the remainder thesis is outlined as follows:

Chapter 2 provides mainly the reviews from previous researchers regarding the use of chemical based mosquito repellents and its drawbacks to human and our environment. To overcome its adverse effects upon its usage on skin, a substitute repellent agent which is more user friendly and environmental friendly is developed from essential oils derived from plant materials. Studies related to its chemical constituents in essential oil (EO) from plant materials are also discussed in this chapter. The techniques involved to separate EO using conventional methods as well as from new technologies are also discussed in detail.

Chapter 3 gives a review mainly on the separation technique using ultrasonic assisted hydro-distillation process in separating EO from plant materials. Essential oil yield collected from *Melissa officinallis* and its mixture with *Cymbopogan citratus* leaves will be compared using three different parameters. Further a brief discussion will be provided on the characterization and repellence test to be done after the separation of EO.

Chapter 4 provides the outcome of the work done using the experimental procedure stated in chapter 3.The analysis which has been conducted will be presented based on three unlike parameters in comparison to maximum yield obtained from the mixture of *Melissa officinallis* and *Cymbopogan citratus.*

Chapter 5 draws together a summary of the thesis and the significant of this research work.

2 LITERATURE REVIEW

2.1 Overview

DEET (N,N-diethyl-3-methylbenzamide) is an effective chemical based repellent against a broad spectrum of insects, and also the most effective and persistence on skin (Isman,2006).Although it is being used world widely, they still do have negative impact which causes environmental and human health risks. Due to the risks associated with DEET, a variety of plant based products have been developed as an alternative to meet the same purpose of DEET. Materials derived from plants could be a source for mosquito repellents agents because they constitute a rich source of bioactive chemicals (Kim et al., 2002).Besides that, they do also possess good efficacy and are environmental friendly. These alternatives to conventional extraction procedures may increase production efficiency and contribute to the protection of the environment by reducing the use of solvents and fossil energy, or generation of hazardous substances. Therefore, an improvise method namely ultrasonic assisted hydro-distillation is applied in this study to increase the yield of oil. Ultrasonic extraction is combined to the conventional method due to its ability to stimulate the release of components localized in the surface glands of the plant material by relatively mild ultrasonic stressing (Toma et al., 2001).

2.2 Introduction

This paper basically presents reviews on the types of mosquito repellents in use currently by users, its drawbacks, alternative repellents derived from plant materials, essential oil, reviews on its bio-actives studies, the extraction methods used to separate oil and its pros and cons.

2.3 Mosquito repellents

Repellents are substances that act locally or at a distance, deterring an arthropod from flying to, landing on or biting human or animal skin (or a surface in general) (Blackwell et al., 2003 ; Choochote et al., 2007).It plays an important role in preventing the transmission of vector-borne diseases by minimizing the contact between human and vectors (Pitasawat et al.,2003; Das et al., 2003).There are two types of repellent that are commercially available and they are namely synthetic chemicals and plant-derived essential oils. The best known chemical based insect repellent is N,N-diethly-3-methyl benzamide (DEET) also known as N,N-diethyle-m-toulamide. DEET is the active ingredients of most commercially available mosquito repellent formulations throughout the world. It is used to repel a variety of animals such as mosquitoes, ticks, flies, gnats and midges. Being DEET not only a broad spectrum repellent, but also the most effective and persistent on skin (Isman, 2006).

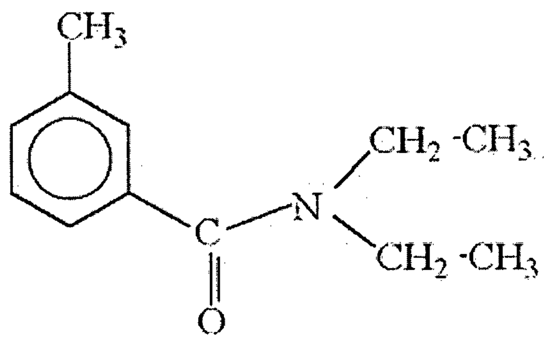

Figure 2.1:DEET

However, widely used synthetic chemical repellents are not safe for humans, especially children, and domestic animals because they may cause skin irritation, hot sensation, rashes or allergy (Das et al.,2003).The side effect after the application of DEET varies from mild to severe. Due to its drawbacks to human health and our ecosystem, more research has been done on repellents that are derived from plant essential oil to replace DEET. Plants may be an alternative source of mosquito repellent agents because they constitute a rich source of bioactive chemicals (Kim et al., 2002). According to Senthil Nathan et al.,(2004), botanical repellents are promising in that they are effective, safe to users, and also inexpensive.

2.4 *Essential oil*

Essential oil referred to as 'essence' are botanical extracts from various plant materials such as leaves, flowers, roots, buds, rhizomes, twigs, heartwood ,resin , seeds and woods. They are natural, volatile, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolite. Commercially, essential oils are used in four primary ways such as pharmaceuticals, as flavour enhancers in many food products, as odorants in fragrances, and as insecticides. Particular emphasis has been placed on their antibacterial, antifungal and insecticidal activities (Chang et al., 2001; Chang & Cheng, 2002).Besides that, bioassays on a number of EO have also shown repellence against mosquitoes (Park et al., 2005, Trongtokit et al., 2005 and Yang et al., 2004) usually attributed to its main compounds and are recorded safe US Food and Drug Administration (Trongtokit et al., 2005).

However, synergistic phenomena between the diverse components of the EO may result in a higher bioactivity (an increased repellent response) of the oil as a whole compared to its isolated components (Hori, 2003). Thus, the fact that an EO contains specific main components may be an indication of its potential use, but does not warrant its use without confirmation of activity. According to Zygadlo and Juliani (2003), their composition may vary considerably between aromatic plant species and varieties, and within the same variety from different geographic areas.

Furthermore, repellent properties of several essential oils are also appeared to be associated with the presence of lower isoprenoids (monoterpenoids, sesquiterpenes and alcohols) (Jaenson et al., 2006). Some monoterpenes such as α-pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are common constituents of a number of essential oils that show mosquito repellent activity (Yang et al., 2004). Among sesquiterpenes, β-caryophyllene is most cited as a strong repellent against *A. aegypti* (Gillij et al., 2008). Although there are several repellent properties of EO regularly appear to be associated with the presence of monoterpenoids and sesquiterpenes (Kiran and Devi, 2007; Jaenson et al., 2006 and Sukumar et al., 1991), a study by Odalo et al(2005) have found that phytol, a linear diterpene alcohol, has high repellent activity against *Anopheles gambiae*. Moreover, the oxygenated compounds phenylethyl alcohol, β-citronellol, cinnamyl alcohol, geraniol, and α-pinene, isolated from the essential oil of *Dianthus caryophyllum*, showed strong repellent activities against ticks (*I. ricinus*) (Tunón et al., 2006).

2.5 Methods of extraction

Extraction is a separation process consisting in the separation of a substance from a matrix. There are several techniques in which separation of EO from plant material can take place. The conventional ways employed to separate EO are hydro-distillation (HD), steam distillation, solvent extraction and also by using Soxhlet. Among these methods HD and steam distillation has been the most common way to extract EO from medicinal herb plants. From studies carried out by Aakanksha et al. (2013), the average percent of yield using hydro-distillation procedure was 0.8% as compared to steam distilled oil (0.7%). Although these techniques have been used since many years for EO's extraction, their application has shown several disadvantages like losses of some volatile constituents, low extraction efficiency, degradation of unsaturated, or ester compounds through thermal or hydrolytic effects, and possible toxic solvent residues in extracts or EO's (Temelli et al.,2007). However, in order to overcome the disadvantages and improve the production yield new extraction technologies such as microwave-assisted extraction(MAE) ,pressurized solvent extraction, supercritical fluid extraction , and ultrasound-assisted extraction has been introduced (Flamini et al.,2007).

2.5.1 Hydro-distillation

Hydro-distillation is one of the most commonly used techniques for volatiles isolation. This method utilises only water to obtain its isolates. In the HD process, the plant material is completely immersed in boiling water. The characteristic feature of this process is that there is direct contact between boiling water and the raw material(Fatima et al., 2014).The isolates namely its essential oil are distillation products that are immiscible with water and can be easily separated. However, some compounds of the EO are water soluble at an elevated temperatures and this may cause some loss in terms of its yield (Halim et al., 2010).

2.5.2 Steam distillation

Most EO's are also obtained from the plant material by a process known as steam distillation. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point of the individual constituents. The process starts when the sample to be extracted is placed in a chamber and hot vapour is allowed to pass through it. Both water and steam are utilized, but the plant material is not indirect contact with water

(Fatima et al., 2014).Heat which is supplied from the hot vapour aids to open the secretary structure which usually contains aromatic compounds and produces essential oil. When the secretary vessels opens up it releases the aromatic compound in the vapour form and rises along with the steam (hot vapour).As the vapour rises up to the condenser it is condensed by the flow of cold water into the condenser. Finally transforming from gaseous to liquid state and will be collected in a collecting funnel. Previous studies have also mentioned that steam distillation is known as one of the most utilized method for obtaining EO's at large scale; however, this technique which used to be considered reasonable for field operation in the past represents as an expensive method, waste of energy and time due to the increase in the cost of energy and the demand at industry for fast processes.

2.5.3 Solvent Extraction

Solvent extraction refers to the distribution of a solute between two immiscible liquid phases in contact with each other (Cox,2004). It is known as one of the simplest and effective techniques to extract EO. Its main disadvantage is contamination of the sample with the solvent (or impurities in the solvent) which needs to be completely removed either to characterize the olfactory qualities of the oil or to study its biological activity (Fatima et al., 2014). Plant material usually cannot withstand high temperature. This is because high sustained heat which is provided can cause the organic components in the plant to decompose and hence the essential oil cannot be extracted. In this process the sample of material is first washed using solvents such as ether, methanol and hexane. This step allows the organic compounds in the plant material to dissolve into the solvent. The solvent mixture is then filtered and distilled under low pressure to attain its EO.

2.5.4 Soxhlet Extraction

Soxhlet extraction is the removal and recovery of organic analytes from a permeable solid matrix by means of a solvent which is continually evaporated from a still-pot and condensed in such a manner that it falls into and permeates through the matrix which itself is held in a permeable container in a siphonable chamber. Soxhlet extraction has been used for over 120 years (since 1879) and is commonly used as a benchmark for total extractable organic residues. The operation of the Soxhlet extractor is intuitively easy to grasp. Extraction process by using this method is viewed to complete or close to complete.

2.5.5 *Ultrasonic-assisted extraction*

Ultrasonic extraction is the removal and recovery of organic analytes from a permeable solid matrix by means of a solvent which is energized by sound energy at frequencies in excess of those audible to the human ear. The application of ultrasound as a laboratory based technique for assisting extraction from plant material is widely published. There are also reviews which have been published in the past to extract plant origin metabolites (Knorr,2003) and bio actives from herbs (Vinatoru (2001). The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave.

During the application of ultrasound, cavitation bubbles are produced and compressed.. The increase in the pressure and temperature caused by the compression leads to the collapse of the bubble. With the collapse of bubble, a resultant "shock wave" passes through the solvent which eventually enhances the mixing (Paniwnyk et al.,2001). Schock waves and powerful liquid micro jets generated by collapsing cavitation bubbles near or at the surface of the sample also accelerates the extraction (Kellner et al., 2004). Moreover, ultrasound also exerts a mechanical effect, which allows greater penetration of solvent into the sample matrix, thus increasing the contact surface area between solid and liquid phase. This coupled with the enhanced mass transfer and significant disruption of cells, via cavitation bubble collapse, increases the release of intracellular product into the bulk medium.

Besides that, according to Paniwnyk et al (2001)and Palma and Barroso (2002)the use of higher temperatures in UAE can also increase the efficiency of the extraction process due to the increase in the number of cavitation bubbles formed. Ultrasonic assisted extraction has many advantages since it can be used for both liquid and solid samples, and for the extraction of either inorganic or organic compounds. The efficiency of the extraction depends on the instrument frequency, and length and temperature of sonication. The benefit of using ultrasonic pre-treatment before extracting oil from seeds of Jatropha curcas L.,and almond and apricot seeds by aqueous enzymatic oil was evaluated by Sharma et al., (2006) and its has shown significantly higher yield with reduction in extraction time.Ultrasonification is rarely applied to large-scale extraction; it is mostly used for the initial extraction of a small amount of material. It is commonly applied to facilitate the extraction of intracellular metabolites from plant cell cultures (Kaufmann, 2002; Sarker, 2006).

2.5.6 Pressurized Solvent Extraction

Pressurized solvent extraction or "accelerated solvent extraction," employs temperatures that are higher than those used in other methods of extraction, and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples.In this extraction process the solid sample will be loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material.High temperatures and pressures increase the penetration of solvent into the material and improve metabolite solubilization, enhancing extraction speed and yield. Moreover, with low solvent requirements, PSE offers a more economical and environment-friendly alternative to conventional approaches. As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity. An additional advantage is that the technique can be programmable, which will offer increased reproducibility(Kaufmann, 2002; Tsubaki, 2010; Sarker, 2006).

2.5.7 Microwave assisted extraction

Microwave-assisted extraction (MAE) or simply microwave extraction is a relatively new extraction technique that combines microwave and traditional solvent extraction.MAE extractive processes are different from those of conventional methods because the extraction occurs as theresult of changes in the cell structure caused by electromagnetic waves. The microwave energy has been investigated and widely applied in analytical chemistry to accelerate sample digestion, to extract analytes from matrices and in chemical reactions.

Application of microwaves for heating the solvents and plant tissues in extraction process, which increases the kinetic of extraction, is called microwave-assisted extraction.According to Perino et al., (2010),the application of microwaves dramaticallyreduces both the extraction time and the volume of requiredsolvent, which automatically aids in reducing environmental burden by diminishing CO2 to the atmosphere. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles, without changing the molecular structures if the temperature is not too high. Nonpolar solvents, such as hexane and toluene, are not affected by microwave energy and, therefore, it is necessary to add polar additives.

Microwave-assisted extraction (MAE) is an efficient extraction technique for solid samples which is applicable to thermally stable compounds accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from materials. The microwave-assisted extraction technique offers some advantages over conventional extraction methods. Compared to conventional solvent extraction methods, the microwave-assisted extraction (MAE) technique offers advantages such as improved stability of products and marker compounds, increased purity of crude extracts, the possibility to use less toxic solvents, reduced processing costs, reduced energy and solvent consumption, increased recovery and purity of marker compounds, and very rapid extraction rates.

2.5.8 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is one of the relatively new efficient separation method for the extraction of essential oils from different plant materials.SFE is a promising technique for industrial application (Reverchon,1997). This emergent extraction technique is usually faster, more selective toward the compounds to be extracted, as well as more environmentally friendly when compared to traditional methods. The new products, extracts, can be used for the production of pharmaceutical drugs and additives in the perfume, cosmetic, and food industries. Use of SFE under different conditions can allow selecting the extraction of different constituents.

The main reason for the interest in SFE was the possibility of carrying out extractions at temperature near to ambient, thus preventing the substance of interest from incurring in thermal denaturation. SFE utilizes the ability of certain gases to behave as nonpolar solvents once acertain temperature and pressure combination has been reached. The most popular gas to be used as a solvent in SFE is carbon dioxide (CO2), because it is nonflammable, noncorrosive, inexpensive,and has generally recognized as safe (GRAS) status. However, extraction by using this technique requires higher investment but can be highly selective and more suitable for food products.

2.6 *Melissa Officinallis*

Melissa officinallis member of the family Lamiaceae (formerly Labiatae) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped . The leaf surface is coarse and deeply veined, and the leaf edge is scalloped or toothed (Turhan, 2006). It is commonly referred to as 'lemon balm' because of its lemon-like flavour and fragrance (Anonymous, 2003). Lemon balm is widely used in herbal medicine and is native to the eastern Mediterranean region and western Asia (Meftahizade and Sargsyan,2010).

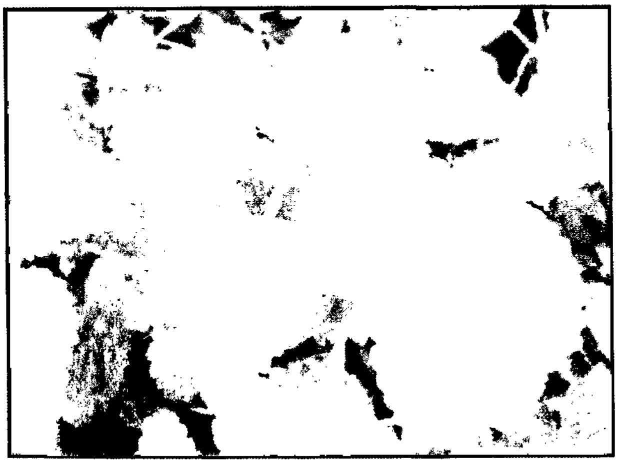

Figure 2.2: Lemon balm

According to Meftahizade et al. (2010), the main constituent of the essential oil are citral (geranial and neral), citronellal, geraniol, beta-pinene, alpha-pinene, beta–caryophyllene, comprising 96% of the oil ingredients. Also Carnat et al. (1998), reported the chemical composition of essential oil of lemon balm, and found that major components are citral representing 48% of the essential oil, followed by citronellal with 39.47% and caryophyllene with 2.37% and in another investigation, the percentage of the main constitute found by Sarer and Kokdil, are alpha-pinene (2.86%), beta-pinene (11.37%), linalool (2.74%), citronella (5.86%) borneol (0.62%), neral (12.22%), and geraniol (38.13%).

Melissa officinalis essential oils have shown notable biological activities including antiviral, antibacterial, antioxidant, antimicrobial activities (Allahverdiyev et al., 2004; Mimica-Dukic et al., 2004). In addition, it has traditionally been employedas a tonic, antispasmodic, carminative, diaphoretic, and sedative-hypnotic for strengthening the memory (Blumenthal et al., 2000). Currently it is used for the relief of stress-induced headaches and as an antiviral to improve the healing of herpes simplex cold sores. According to Farook et al. (2013), lemon balm also helps to improve cognitive function and decrease agitation in patients with Alzheimer's disease. It has also been reported to possess insect repellent properties (Sharafzadeh et al., 2011) due to the presence of citronella in its chemical constituents.

2.7 Cymbopogan Citratus

Cymbopogan citratus is a herb plant which belongs to the family Poaceae is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Oceania. *Cymbopogon citrates*, commonly known as lemongrass and other Cymbopogon species is a tall, clumped aromatic perennial coarse grass that can grow up to 1 m height. The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm (Sugumaran et al., 2005). Besides that, lemongrass is also known as barbed wire grass, silky heads, citronella grass or fever grass amongst many others.

Figure 2.3: Lemongrass

Essential oils are natural products obtained from plants. They were formed by varied and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which were deposited in various structure of the plant (Linares et al., 2005).Fresh lemongrass contains approximately 0.4% volatile oil and rest of it are non- volatile components and nutritious such as calcium, iron, magnesium, manganese, phosphorus, potassium, selenium and zinc. Its oil has a lemony, sweet smell and is dark yellow to amber and reddish in colour with a watery viscosity.It constitutes mainly citral. Citral of lemongrass is a natural combination of two isomeric aldehydes, namely isomers geranial (α- citral) and neral (β- citral) (Pengelly,2004). According to Schaneberg and Khan,(2002) other unusual active components of the EO are limonene, citronellal, β-myrcene and geraniol.